# Improvement of seed germination of *Magnolia grandiflora L*

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**Citation**
Ivanova, V., Nacheva, L., & Genova, D. (2019). Improvement of seed germination of *Magnolia grandiflora L. Rastenievadni nauki, 56*(5), 12-18

**Abstract**
The distribution of genus *Magnolia* is limited due to difficulties in breeding and production of propagating material. The aim of present investigation is to increase the germination of *Magnolia grandiflora L.* seeds. The seeds were collected in September 2017, from a 15-year-old tree in Plovdiv region. After 2 months they were divided into 2 groups - half of them remained dry at room temperature and the other half was disinfected following the standard procedure for the Laboratory of "Plant Biotechnology". The disinfected seeds were divided into 2 subgroups in sterile glass jars with sterile wet perlite in variants at temperatures of 4 °C and 22 °C in the dark for 70 days. Gibberellic acid (GA3), Biolan and Agrostimulin were tested for germination stimulation. It was found that stratification at low positive temperature is a sine qua non for seed germination of studied magnolia. The results showed that treatment of seeds of *Magnolia grandiflora* L. with growth regulators does not increase seed germination under the specific conditions, but affects the further development of seedlings. Seeds stored at room temperature did not germinate under the conditions of the study, regardless of treatment with growth regulators. Plants obtained from 0.02% Biolan-treated seeds had higher fresh leaf weight and larger leaf area than the other variants. Treatment of the seeds after stratification with 0.005% Agrostimulin had positive influence on the development of the root system.

**Keywords**: *Magnolia grandiflora L.*; propagation; seeds; growth regulators.

## INTRODUCTION

Magnolias belong to the family *Magnoliaceae* and are considered to be the oldest flowering plants. The genus Magnolia covers about 80 species of trees that are naturally distributed in the East of North America and South East Asia (Nooteboom, 1993). Types of magnolia are widely grown as ornamental plants (Dirr, 2002). Their leaves and colors are highly valued, and their fruits are a great food for wildlife (Callaway, 1994). Over 2% of the wood extraction in the South=Eastern US is from magnolia. *Magnolia grandiflora L.* (Magnolia, Northern Magnolia) is spread in the southern parts of North America. It was transported to Europe in 1731 (Nooteboom, 1993). It can withstand short-term cold conditions up to -18 degrees. In our country, magnolia is found in areas with a warmer climate such as the Black Sea Coast and the southern parts of the country (Strandzha, Petrich, etc.). *Magnolia* spp. are also successfully grown in the area of Plovdiv. Because of their beautiful crown and beautiful large flowers magnolias are used in landscape design in parks for single or group planting and are increasingly preferable for private gardens and yards *Magnolia grandiflora L.* is particularly suitable for urban landscaping because of its resistance to dryness, to sulfur dioxide and acid deposition (Halls, 1977; Outcalt, 1990; Gilman & Watson, 1993b). The aim of the current study was to improve the germination of *Magnolia grandiflora L.* seeds.

## MATERIAL AND METHODS

Laboratory experiments were held at the Fruit Growing Institute, Plovdiv. The seeds for the present study were collected from about 15 years old tree *Magnolia grandiflora L.*, growing in the region of Plovdiv. The tree is very well developed, with a height of about 7-8 meters. The seeds were harvested in September 2017, cleaned and dried at room temperature in a dry room and stored in a paper bag in which they could breath and never got bloated. After 2 months the seeds were divided into 2 groups - half of them remained dry stored at room temperature and the other half was disinfected according to a standard procedure for the Plant Biotechnology Laboratory:

1. Wash with running water and liquid soap for 30 minutes;
2. Seed treatment for 30 seconds with 95% ethanol;
3. Rinse with sterile distilled water;
4. Treatment of seeds with 5% solution of calcium hypochlorite for 7 minutes;
5. A triple rinsing with sterile distilled water for 10 minutes.

The in this way disinfected seeds were divided into 2 subgroups in sterile glass jars with sterile wet perlite.

1. At temperature of 4 °C in the dark (in the fridge) - 300 pieces;
2. At temperature of 22 °C - in the dark (in the phytostatic chamber) - 50 pieces.

70-day seeds have been removed from the jars with the perlite and cleaned from the sheath. Seeds stored at room temperature in paper bags during the entire period, were disinfected by the above procedure. To stimulate the germination of the seeds are tested Gibberellic acid (GA3), Biolan and Agrostimulin. The seeds treated in the cold and warm are treated with growth regulators which includes the following options (Table 1).

For each of the listed options, 50 seeds are planted. After treatment, accordingly, the seeds are seeded on variations in peat-perlite mixture (2:1). They are coated with a thin layer of perlite and a polyethylene foil to maintain humidity (Figures 6, 7). They were left in a growth chamber at 22 ± 2 °C and 16/8 hours photoperiod (60 μmol $m^{-2}$ $s^{-1}$ PHAR, 40W OSRAM white fluorescent lamps). The germination is recorded in dynamics from 35 days to 150 days (the appearance of the first germinated seed).

On the 150th day the seedlings were carefully transferred to seedlings with the same peat-pearl mixture and exported outward under a black mesh. Non-sprouting seeds were left in the growth chamber. Biometric indicators - fresh and dry mass of individual botanical organs - leaves, stems, roots, stem height, number of leaves, leaf area of the plant, number of roots and branches, root length, root system volume reported at the end of July

**Table 1.** Variants of treatments

| Variant | Temperature, °C | GA3, ppm | Biolan, μl/l | Agrostimulin, μl/l |
|---|---|---|---|---|
| **1 (Control 1)** | 4 | | | |
| **2 (Control 2)** | 22 | | | |
| **3** | 4 | 2500 (12 h) | | |
| **H3** | 22 | 2500 (12 h) | | |
| **4** | 4 | | 100 (12h) | |
| **H4** | 22 | | 100 (12h) | |
| **5** | 4 | | 200 (12h) | |
| **H5** | 22 | | 200 (12h) | |
| **6** | 4 | | | 50 (8h) |
| **7** | 4 | | | 50 (12h) |
| **H7** | 22 | | | 50 (12h) |

2018. For each variant, 5 plants were measured. The results obtained were statistically analyzed by variance analysis (ANOVA), and the significance of differences between variants was assessed by a Duncan's multiple range test, P <0.05).

## RESULTS AND DISCUSSION

The analysis of the results of this study showed that treatment with low positive temperatures in humid environment is a necessary condition for the germination of seeds of evergreen magnolia (*Magnolia grandiflora L.*) (Fig. 1, 2). All seeds stored at room temperature (variants 2, H3, H4, H5 and H7) did not germinate under the conditions tested. Regardless of the treatment with gibberellins (variant H3) and bio analysts Biolan (H4, H5) and Agrostimulin (H7), the percentage of germinated seeds was negligible (less than 3%).

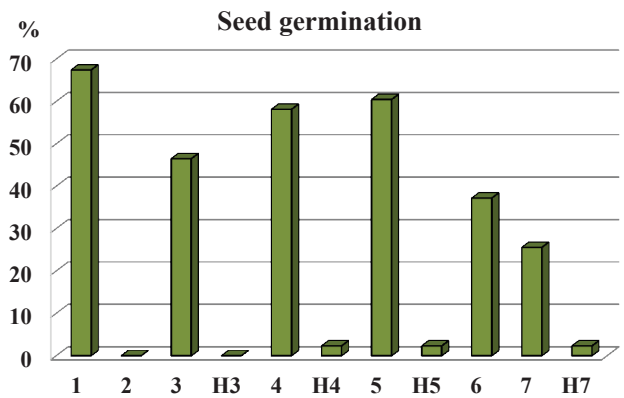


**Figure 1.** Seed germination (%) of *Magnolia grandiflora* L.

Seeds treated with low temperature (4 °C) have a germination between 25 and 67% - significantly higher compared with those stored at room temperature (Figure 1). The highest percentage of germination (67%) was reported in variant 1, followed by variants 5 and 4 by 61% and 58%, respectively.

The first seeds germinated 35 days after pledging their substrate warm. Interesting is the dynamics of germination of the different variants (Fig. 2.). At jointly, within 3 days germinated seeds from the control version 1. All other variants treated with growth regulators, germinated in a longer period of time, while most of them were observed 2 or 3 peak - first - between 35 and 38 day, followed by a peak about the 45th day and the third at the 56th day. This is probably related to the endogenous sources of growth regulators.

According to Ibrahim et al. (2010), seed treatment with GA3 and IAA alone or in combination results in significant increase in germination and stimulates the further development of seedlings as compared to untreated controls. Optimal results were obtained in pre-sowing treatment with a combination of the two growth regulators at the highest concentration used - 2500 ppm, with a germination of 66.25% of the seeds, 8.53 cm stem height, 68.84

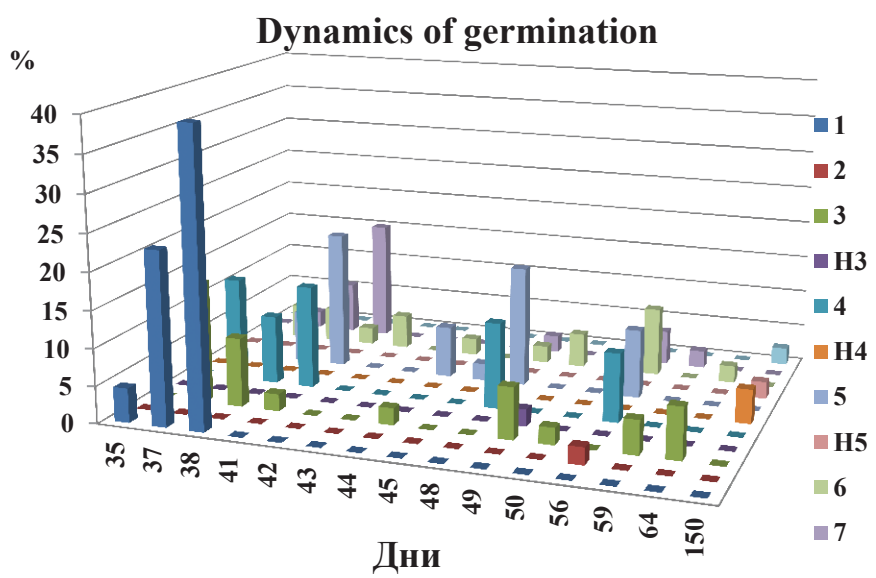


**Figure 2.** Dynamics of germination of seeds from 35 to 150 a day

cm$^2$ total leaf area of one plant and 3.30 cm$^3$ volume root system. There were no significant differences between the variations with respect to the average leaf area.

In our study, seed treatment with 2500 ppm GA3 (one of the optimal variants according to the above author) did not result in the expected increase in germination. In this variant seed germination was 47% and was lower than the control. However, high germination in the control variant is noteworthy. In the studies of Ibrahim et al. (2010) untreated seeds had 30% germination (the seeds are stored in a refrigerator at 1-5 °C dry in paper bags). In our experiment, stratified seeds are stored at the same temperature but in a moist perlite, and this is probably the reason for their significantly higher germination rate. Again, in wet perlite, but at 22 °C (lime 2) there were no sprouted seeds.

Our results are in accordance with the reports of the effects of treatment with growth regulators to seeds of other species - *Ginkgo biloba* (West et al., 1970; Johnson & Wickliff, 1974) *Cassia obtusifolia* (Singh & Murthy, 1987; Morus, 2006), *Pyracantha crenulata* (Joshi et al., 2010), *Picea smithiana* (Singh, 1990), *Azadirachta indica* (Sivgnanam, 1995), *Nigra* (Koyuncu, 2005), *Abies pindrow*, *Cupressus torulosa* and *Picea smithiana*.

The treatment of the seeds of *Magnolia grandiflora L.* with growth regulators does not enhance germination of seeds under the particular conditions, but affects the further development of seedlings. The plants obtained from 0.02% Biolan-treated seeds have a higher fresh leaf mass (Fig. 3) and a larger leaf area compared to the other variants (Fig. 6). No significant difference was found in the average number of leaves among stratified variants at low positive temperature.

The highest value of the fresh stem mass was recorded in the seed treatment variant with 0.005% Agrostimulin for 12 hours (Fig. 7).

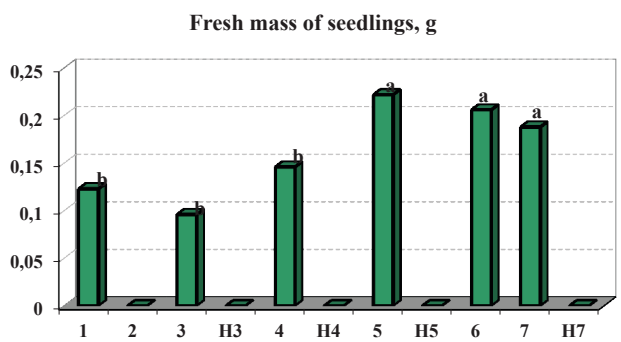


**Figure 3.** Fresh mass of seedlings of *M. grandiflora* L., g

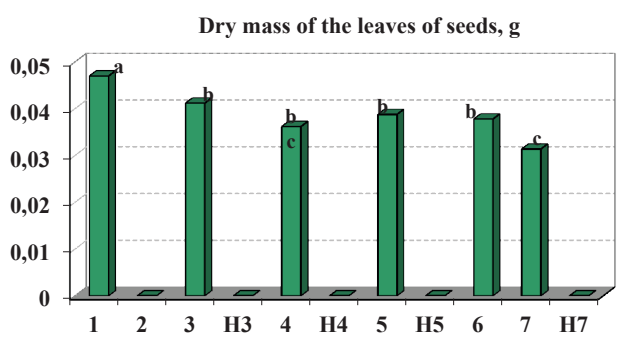


**Figure 4.** Dry mass of the leaves of seeds of *M. grandiflora* L., g

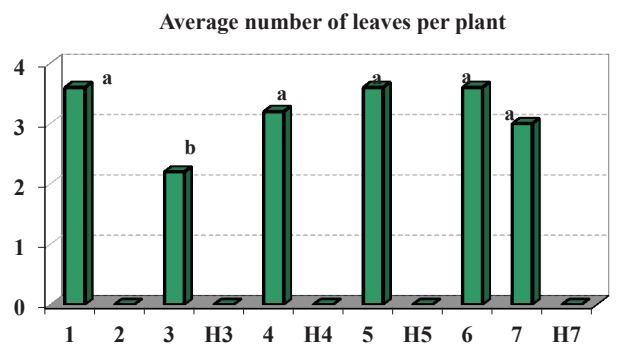


**Figure 5.** Average number of leaves per plant

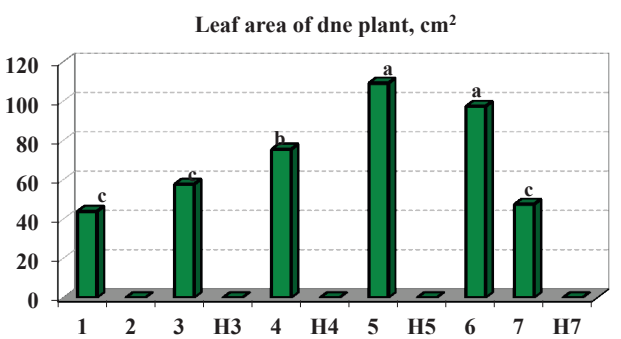


**Figure 6.** Leaf area of one plant, cm $^2$

The analogous treatment with a shorter exposure (6 hours) is distinguished by lower fresh mass, dry mass and stem diameter (Fig. 7, 8, 9), but the statistical difference is only confirmed by the first indicator. Agrostimulin treatment also positively affects the development of the root system. In both seed treatment variants, the highest root mass (11), the largest volume of the root system (Fig. 13) and most root branches (Fig. 14).

There are no significant differences in the height of the stem from the different treatments.

The results of the conducted study show that stratification at low positive temperature in a humid environment is a necessary condition for the proper development of embryos and seed germination. Although growth regulators in our tested concentrations and exposures do not increase seed germination, they have a beneficial effect on the further

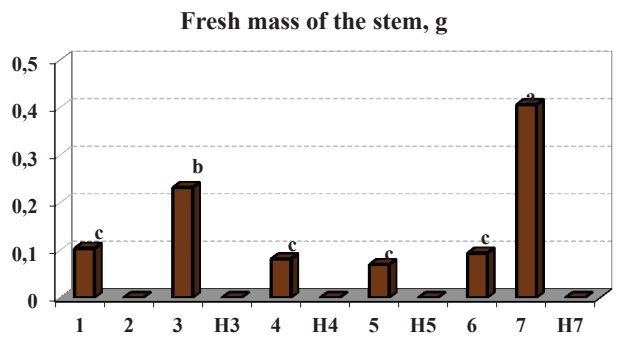


**Figure 7.** Fresh mass of the stem, g

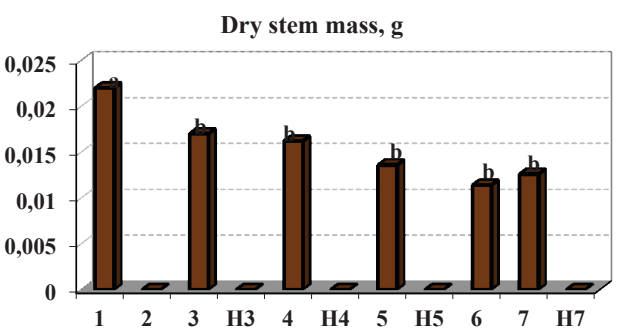


**Figure 8.** Dry stem mass, g

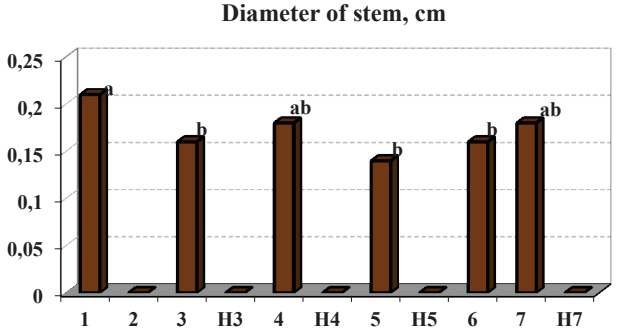


**Figure 9.** Diameter of stem, cm

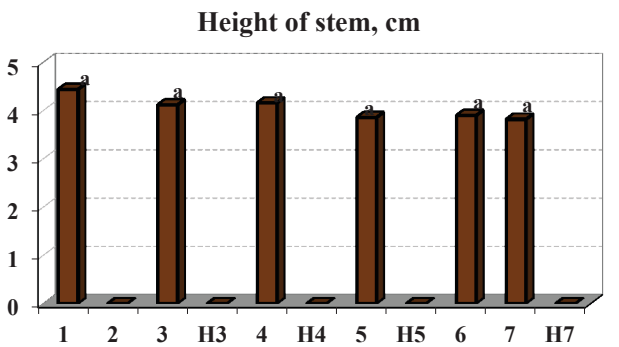


**Figure 10.** Height of stem, cm

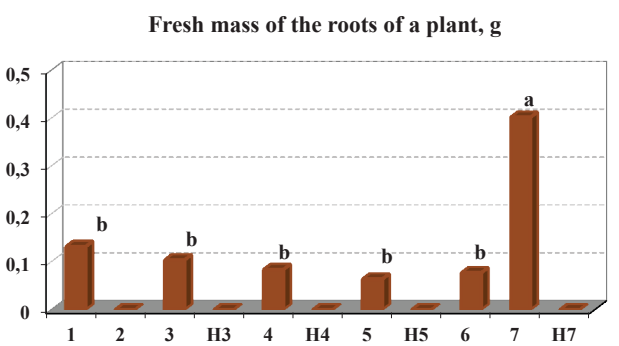


**Figure 11.** Fresh mass of the roots of a plant, g

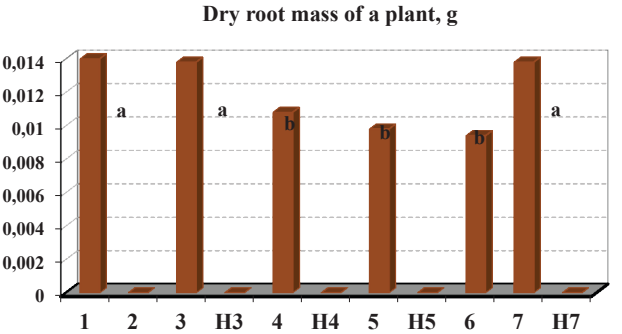


**Figure 12.** Dry root mass of a plant, g

development of seedlings. These results are in line with those obtained from Kumaran et al. (1994) on the stimulant effect of growth regulators (IAA) on *Azadirachta indica* not only on seed germination but also on the further development of seedlings.

Further research is needed to optimize the presowing treatment of *Magnolia grandiflora L.* seeds with regard to the type and mode of action of different growth regulators. Establishment of effective seed treatment protocols to achieve higher germination and also for better development of seedlings would contribute to improving the seed propagation of evergreen magnolia.

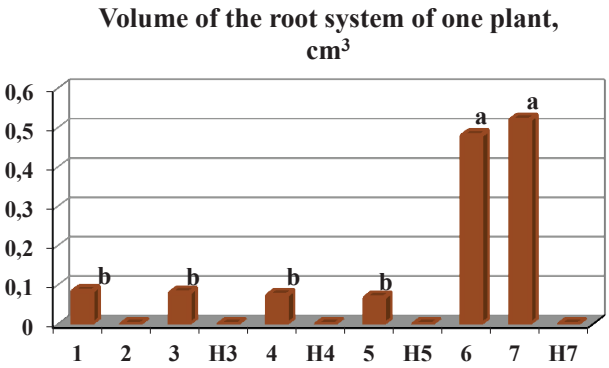


**Figure 13.** Volume of the root system of one plant, $cm^3$

## CONCLUSIONS

1. Stratification at low positive temperatures in humid environment is a necessary condition for the germination of seeds of evergreen magnolia (*Magnolia grandiflora* L.). All seeds stored at room temperature (variants 2, H3, H4, H5 and H7) did not

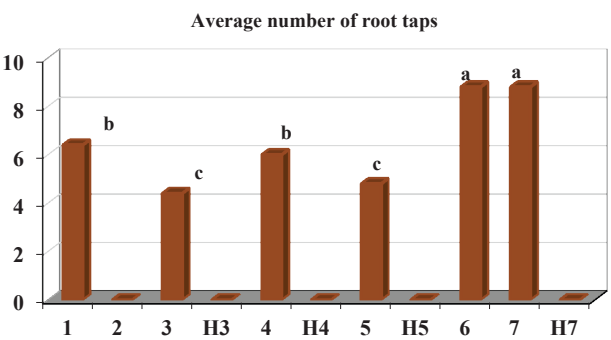


**Figure 14.** Average number of root taps

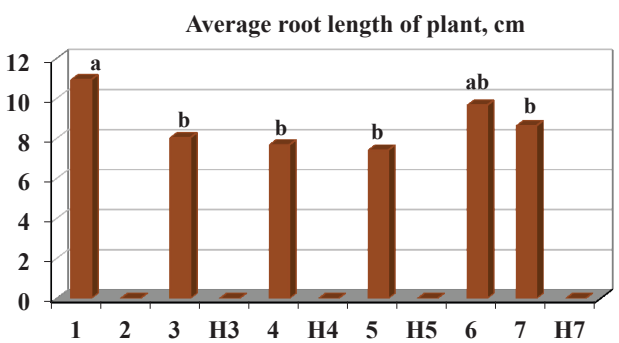


**Figure 15.** Average root length of plant, cm


**Figure 16.** General view of *Magnolia grandiflora L.* seedlings

germinate under the conditions of the current study, regardless of treatment with Gibrebrelinic acid, Biolan or Agrostimulin.

2. The treatment of the seed of *Magnolia grandiflora* L. with growth regulators does not enhance germination of seeds under the particular conditions, but affects the further development of seedlings.

3. Plants obtained from 0.02% Biolan-treated seed have a higher fresh leaf weight and a larger leaf area than the other variants.

4. Treatment of the seeds of a large-scale magnolia after stratification with 0.005% Agrostimulin has a positive influence on the development of the root system. In both versions (6 and 7) is recorded and the highest value of the fresh weight of the roots, the largest volume of the root system and most root branching.

## ACKNOWLEDGEMENTS

Biolan and Agrostimulin were kindly provided by Academic Serhiy Ponomarenko (Agrobiotech, Ukraine) as a part of the bilateral Bulgarian-Ukrainian project DHTC/Ukraina/01/9/2012, supported by National Science Fund, Ministry of Education and Science, Bulgaria.

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